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Regular research paper

Krystyna FALIŃSKA<sup>1</sup>, Marlena LEMBICZ<sup>2</sup>, Artur JARMOŁOWSKI<sup>3</sup>, Lidia BORKOWSKA<sup>4</sup>

<sup>1</sup> W. Szafer Institute of Botany Polish Academy of Science, Lubicz 46, 31-512 Kraków, Poland and Próchnika 8b/140, 01-585 Warsaw, Poland, e-mail: k.falinska@chello.pl (corresponding author)

<sup>2</sup> Department of Plant Taxonomy, A. Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland

<sup>3</sup> Department of Gene Expression, A. Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland

<sup>4</sup> Department of Botany, Department of Botany, University of Podlasie, B. Prusa 12, 08-110 Siedlce, Poland

## PATTERNS OF GENETIC DIVERSITY IN POPULATIONS OF *FILIPENDULA ULMARIA* (L.) AT DIFFERENT STAGES OF SUCCESSION ON A MEADOW ABANDONED FOR 30 YEARS

**ABSTRACT:** Genetic diversity and spatial variation of two populations of *Filipendula ulmaria* (L.) were analysed at the initial stage (macroforbs) and the late succession stage (forest community) during a 30-year succession on the abandoned meadows in Białowieża National Park. The study demonstrated a high level of genetic diversity in both populations ( $D = 0.86$  and  $D = 0.79$  in the population at the initial and late succession stage, respectively). Significant genetic differentiation was observed at the cross-population level ( $F_{ST} = 0.008$ ,  $P < 0.001$ ). Among 303 ramets in the population at the initial stage 261 genotypes were detected, of which 24 were clonal, and the remaining (237) were unique (91%). In the population at the late succession stage 92 genotypes were detected among 116 ramets of which 10 were clonal, and 82 unique (89%).

The results did not confirm a hypothesis that the low level of the population's genetic diversity should be expected at the late succession stage. Additionally, the study demonstrates that the prolonged clonal spreading does not always result in genetic monotony of a population. It has been demonstrated that the high level of genetic diversity in the population of clonal species *F. ulmaria* could be the result of temporal changes in life history traits of long-lived genets. The formation of gaps caused by the disintegration of senile genets and the permanent recruitment of seedlings over 30 years of population development reinforce the appearance of new genets. This process was

reflected in both populations by the domination of unique genets and spatial pattern of genotypic diversity.

**KEY WORDS:** genetic variation, genet identification, genotypic diversity, life history, succession.

### 1. INTRODUCTION

The majority of perennials have the ability of sexual and asexual reproduction. Despite the dominance of vegetative reproduction, monoclonal populations have rarely been recorded in populations of clonal species (Kroon and Groenendael, eds. 1997, Eckert 1999, 2002). Low levels of genetic diversity have most frequently been recorded in small, island and endangered populations (Brzosko *et al.* 2002, Brzosko and Wróblewska 2003, Izawa *et al.* 2007, Czarnecka and Ptaszyńska 2008, Kostrakiewicz and Wróblewska 2008). Usually, populations of clonal plants are formed by a few genets, especially in patched distribution pattern, such as found in *Anemone nemorosa* (L.) (Stehlik and Holderegger 2000), *Maianthemum bifolium* (L.) (Honnay *et al.* 2006) and *Vaccinium stamineum* (L.) (Kreher *et al.* 2000), as a results of the spreading

modes of clones and repeated seedling recruitment pattern (RSR) (Eriksson 1989, 1993, 1997). The spatial-temporal variability of the two modes of reproduction is responsible for the development of monoclonal and multiclinal patches in mono-species communities (Stehlik and Holderegger 2000, Kreher *et al.* 2000, Person and Gustavsson 2001, Honnay *et al.* 2006). The formation of monoclonal populations is, predominantly, explained by the prolongation of clonal spreading and/or inhibited flowering and fruiting of long-lived genets. Additionally, various ecological factors, especially those limiting the recruitment of seedlings by, for example, low production of seeds or their reduced germination potential (Eckert 2002) may affect the genetic diversity of clonal plant populations. This could be the result of increased shading (Slade and Hutchings 1987) caused by, for example, a canopy of tree developing during succession, which reduces fruiting and seedling recruitment (Stehlik and Holderegger 2000, Torimaru *et al.* 2003).

Results of previous studies indicate that the level of genetic diversity in populations of clonal species is an outcome of a balance between asexual and sexual reproduction, which varies greatly between species, as well as among populations of the same species (Eckert 1999, 2002, Honnay *et al.* 2006). It has been suggested that investigation of the mechanisms determining genetic diversity of clonal plant populations would certainly benefit from the identification of population age. Numerous observations and long-term studies on the dynamics of clonal plant populations indicate that genetic variation is accumulated throughout the population's life (Escaravage *et al.* 1998). However, temporal changes of spatial patterns of genets/clones, which certainly play a significant role in the development of genetic diversity of populations, have been insufficiently studied (Eriksson 1993, Kudo *et al.* 1999). Long-term studies of the dynamics of *Filipendula ulmaria* population during succession on abandoned meadows demonstrated a relation between the longevity of genets and their integration and disintegration, as well as between the dying of senile parts and repeated seedling re-

cruitment (Falińska 1995, 2003). Harnett and Bazzaz (1985) observed a decrease in the abundance of *Solidago canadensis* population in the first 3 years after the formation of a population described by the ISR recruitment pattern, when the population consisted of a few genets. Similarly, *Ranunculus repens* populations were formed by a few genets, since only 3% of early seedlings survived (Watkinson and Powell 1993). In a population of *Anemone nemorosa* described by the RSR pattern, with a seedling survival rate of 3–5%, a high level of genetic diversity was observed at a late succession stage (Stehlik and Holderegger 2000). However, temporal changes and its influence on genetic variability of clonal plant population have been poorly studied, and population age has rarely been identified. The main objective of this study was to investigate the pattern of genetic diversity in populations of *Filipendula ulmaria* over a long-term succession. We test the hypothesis that the patterns of population's genetic diversity at the initial (macroforbs) and late succession stages (forest). Therefore, we analyzed two scenarios of changes in genetic diversity of the populations of *Filipendula ulmaria* over 30-years' succession:

- 1) Along with the progress in succession the level of genetic diversity of the population is reduced because long-lived multi-ramets genets fill in the space, thus limiting the development of seedlings;

- 2) Genetic diversity of the population is maintained through succession stages because of the simultaneous disintegration of senile genets and development of new genets which emerge in gaps among fragments.

The study objective was to address the following detailed questions:

- 1) Whether, and to what extent, the change in the vegetative spreading pattern in long-lived genets influences the level of genetic diversity of the population.

- 2) Whether the seedling recruitment pattern (RSR) is maintained along a 30-year development of the populations.

- 3) Whether, in long-lived clones, the seedling development is reduced as a result of the increased density of ramets and the accumulation of the necromass from the dying parts of genets.

## 2. MATERIAL AND METHODS

## 2.1. Subject of the study

A long-term study (1972–2002) on the dynamics of clonal species populations during succession on an abandoned meadow (15 ha) was carried out in Białowieża National Park, in the valley of the Narewka river (Falińska 2003). These populations are located between the river and forest area. At the initial stage of succession on a wet and floristically rich meadow, specimens of the majority of species were distributed randomly or in clusters. The spatial pattern of clonal species population during succession from the meadow to forest transformed from a random distribution, through clustered to patched pattern. After 30 years, a mosaic spatial pattern of vegetation cover was formed, in which large-surface patches were dominated by clonal species. *Filipendula ulmaria* represented one of these species, and at various succession stages it either promoted or inhibited the transformation of the plant cover. After 30 years the patches of vegetation cover represented different succession stages. *F. ulmaria* occurred in macroforbs (initial stage) and forest (late succession stage). Permanent plots were located in these patches, where then research of the dynamics of clonal species population was carried out for 30 years using the charting method (mapping).

## 2.2. Study populations and sampling

*Filipendula ulmaria* is a long-lived perennial described by a guerrilla growth strategy and two modes of reproduction: asexual and sexual. This species occurred sporadically, on a mown fertile wet meadow (*Cirsietum rivularis*), only on specific sites – the riverside and the margin between the meadow and the forest. After mowing ceased, it occupied, after a short time (9–10 years), a considerable part of the 15 ha meadow, forming patches of different size (200–600 m). After 15–20 years *F. ulmaria* was present on 75% of the meadow area. The population had a patched-clustered pattern of distribution. Patches were formed by large clones (1.2 m), which underwent disintegration during that time, and new genets developed in the created gaps (Table 1). In that period patches of shrubs, trees and sedges were formed on the meadow, and *F. ulmaria* was present between the patches dominated by these species. After 30 years, a significant proportion of *F. ulmaria* was observed only in macroforbs which at that time occupied 0.6 ha. Two populations of *F. ulmaria* were selected for the analysis of genetic diversity. One was located in macroforbs, and another in a small-sized patch (100 m<sup>2</sup>) in a forest community which had been formed in the meadow over 30 years.

Populations selected for molecular analysis, A – in the macroforbs patch, i.e. initial

Table 1. Analysis of spatial distribution of genets and ramets in *Filipendula ulmaria* population in abandoned meadow for 30 years (1976–2006). Data collected from 25 m<sup>2</sup>.

Phase of population dynamics	Age (years)	No. of genets		No. of ramets	Mean per genet M	<i>d</i>	No. of seedlings per 25 m <sup>2</sup>
		M	J				
1 – settlement	5	23	29	115	4.2±0.8	0.89	153
2 – increase	10	24	56	412	17.2±9.8	9.85	173
3 – fluctuation	15	25	201	572	22.9±8.2	17.32	681
4 – maximal	20	*	213	972	37.1±8.7	12.04	191
5 – decrease	25	*	162	290	*	3.00	157
# – 6 – decline	30	*	*	303	*	3.10	104

*d* < 1 – tendency to a uniform distribution; *d* = 1 – random distribution; *d* > 1 – clumped distribution tendency to aggregated (Morisita – dispersion coefficient); # – 2006yr – collected of ramets for genetic analyses; \* – failed data. Genets: juvenile (J), mature (M); 1–5 – source: Falińska (1996, 2003), supplemented.

stage, and B – in the forest community patch, i.e. late succession stage, were located within a 450 m distance. From each population all ramets within plots of 25 m<sup>2</sup>, divided into 1 m<sup>2</sup> squares, were sampled in June, 2007, where, previously, long-term observations had been carried out on all marked genets (Falińska 1991, 1995, 2003). 303 ramets were sampled from population A, and 116 ramets from population B. Maps of the ramets' distribution were prepared prior to their sampling for laboratory procedures.

### 2.3. Laboratory procedures

Samples were collected from the forest and meadow populations of *Filipendula ulmaria* in the summer 2006. At each site an area of 25 m<sup>2</sup> was marked and all ramets of *Filipendula ulmaria* were harvested within the area. Studied materials were young leaf fragments. Each collected sample was placed in a separate Plant material was transported in portable refrigerators to the laboratory.

### 2.4. DNA isolation

Tissue samples, approximately 100 µg weight, were used for DNA isolation. Total genomic DNA was isolated using the Genomic Mini AX Plant kit (AA Biotechnology), following the standard protocol.

### 2.5. AFLP analyses

The AFLP protocol was used in this study. Total genomic DNA (~400 ng) was used for the restriction and ligation reaction with TruI1 and EcoRI restriction enzymes (Fermentas), and T4 ligase (New England Biolabs). Each PCR reaction was performed in a final volume of 25 µl. The preselective amplification was performed with primers with a single selective nucleotide: Eco-1-A and Mse-1-A. The PCR program was as follows: 2 min at 72°C, 30 cycles of 30 s denaturing at 94°C, 30 s annealing at 56°C and 2 min extension at 72°C. The reaction was stopped by 10 min incubation at 60°C. The selective amplification was carried out with four selective primers (ATG/AGC/

TCA/AGG) to generate markers between 70–400 bp in size. All EcoRI selective primers were marked with one of the fluorescent dyes: VIC, PET, NED or FAM. The PCR profile was as follows: 50 s at 94°C, 1 min at 65°C, RAMP 65°C–57°C; 0,7°C/sec, 72°C, then 20 cycles of 50 s denaturing at 94°C, 30 s annealing at 56°C and 2 min extension at 72°C. At the end the samples were incubated for 10 min at 72°C. The PCR products were separated using the ABI 3130XL automated sequencer (Applied Biosystems) with the GeneScan – 600 LIZ internal size standards. Electropherograms were analysed by GeneMapper version 3.7 (Applied Biosystem). The intensity of each peak was normalized on the total signal intensity and the peak was considered only when a fixed threshold was exceeded. The presence and absence of peak were scored in a specific locus. The AFLP fragments were treated as present or absent.

### 2.6. Data analysis

AFLP sequence analysis was carried out using Gene Mapper software, to identify *F. ulmaria* genets and to assess the genotypic diversity and genetic differentiation of the population at the initial (A) and late (B) stages of succession based on the frequency of haplotypes detected in 303 ramets in population A and in 116 ramets from population B.

The analysis of molecular variance (AMOVA) was used for the assessment of genetic diversity within the population and genetic differentiation between populations. The AMOVA was based on haplotypes frequencies and using the conventional *F* statistics were calculated by the method of Weir and Cockerham (1984). Levels of among-populations genetic differentiation ( $F_{ST}$ ) were calculated *F* statistic was based on pairwise genetic distances of populations, according to Weir and Cockerham (1984) using GENEPOP (version 1.2). (Raymond and Rousset 1995). Genetic diversity (*D*) in each population was calculated according to Ellstrand and Rose (1987) as: the proportion of distinguishable genets (*G/N*) with *G* the number of genets and *N* number of ramets.

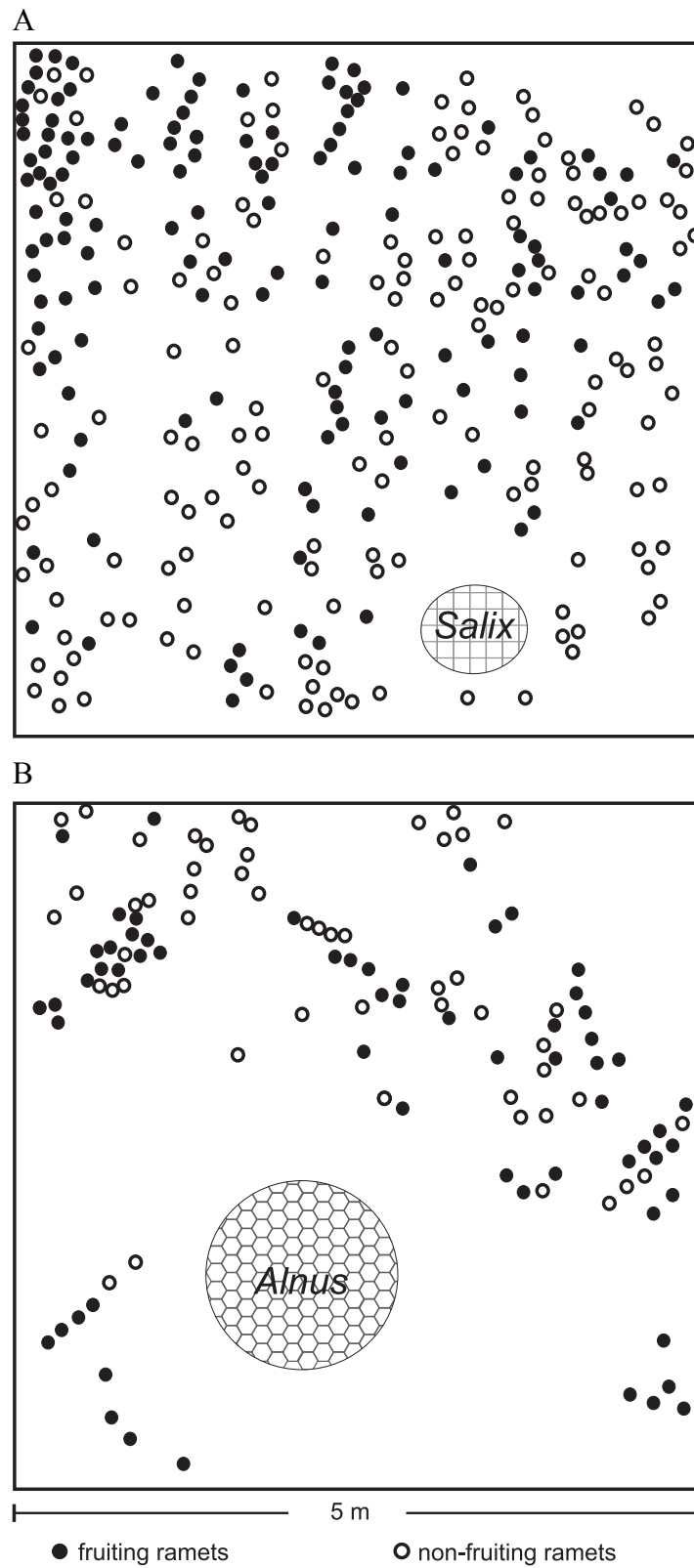


Fig. 1. Spatial pattern of *Filipendula ulmaria* populations at initial (A) and late (B) succession stage. Each symbol corresponds to a single genotype. Data collected from plot  $5 \times 5$  m. The occurrence of *Salix* and *Alnus* is given by circles.

## 3. RESULTS

## 3.1. Spatial pattern of populations

Populations of *F. ulmaria* at the initial (A) and late (B) succession stages differ in spatial abundance and distribution of ramets. Population A was almost 3 times larger than B when the numbers of ramets were compared. The two populations also differed in spatial pattern, which in the population of macroforbs (A) could be described as a mixed model, because of the almost uniform distribution of ramets occupying the whole plot, but in the area covered by this aggregation small few-ramet clusters were distinguished. The forest population (B) was characterized by a clustered pattern. Ramets of *F. ulmaria* occupied about a half of the plot, demonstrating a tendency to concentrate around or between the trees (Fig. 1) In both populations the proportion of fruiting ramets was similar e.g. at the initial stage it amounted to 55%, and to 45% at the late stage of succession.

## 3.2. Genotypic diversity and genetic variation within and between populations

Among 303 ramets growing in macroforbs at the initial stage (A), 261 genotypes were identified, of which 237 ramets (91%) were unique. In the forest community, i.e. the late succession stage (B), among 116 ramets 92 genotypes were identified, of which 82 (89%) were unique. Altogether, 34 few-ramet genets (clones) were identified and 14% of them (5 genets) were present in both populations. The level of genotypic diversity, calculated as the ratio of distinct genotypes number (G) to number of sampled ramets (N), was high in both populations ( $D = 0.86$  in population A,  $D = 0.79$  in population B (Table 2)). The analysis of gene diversity revealed high values in both populations ( $0.9983 \pm 0.0006$  for A, and  $0.9859 \pm 0.0062$  for population B).

Results of the analysis of molecular variance (AMOVA) suggest that genotypic variation within a population is higher than between populations (percentage of variation 99.22 and 0.78, respectively). However,

Table 2. Number of genotypes *G* and ramets *N* and ratio of genetic diversity *G/N* in populations of *Filipendula ulmaria* at initial (A) and late (B) succession stage.

Populations	Number of genotypes <i>G</i>				$\Sigma$	No. of ramet <i>N</i>	<i>D</i> <i>G/N</i>	No. of ramets in genet <i>G</i>				$\Sigma$	Mean <i>N</i> per genet		
	with ramets		unique					2	3	4					
	<i>n</i>	%	<i>n</i>	%											
A	24	9	237	91	261	303	0.86	12	50	7	28	5	22	24	2.8
B	10	11	82	89	92	116	0.79	6	60	2	20	2	20	10	3.4

Table 3. Analysis of molecular variation (AMOVA) in the population at initial and late succession stage for *Filipendula ulmaria*.

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among populations	1	1.155	0.00392Va	0.78
Within populations	417	207.439	0.49746	99.22
Total	418	208.594	0.50138	

Table 4. Number of genotypes present in both populations of *Filipendula ulmaria* at initial (A) and late (B) succession stage.

Genotypes marked	Number of ramets		Sum of ramets (copies)
	A	B	
6	8	13	21
10	3	1	4
12	2	1	3
13	1	3	4
14	1	1	2
$\Sigma$	15	19	34

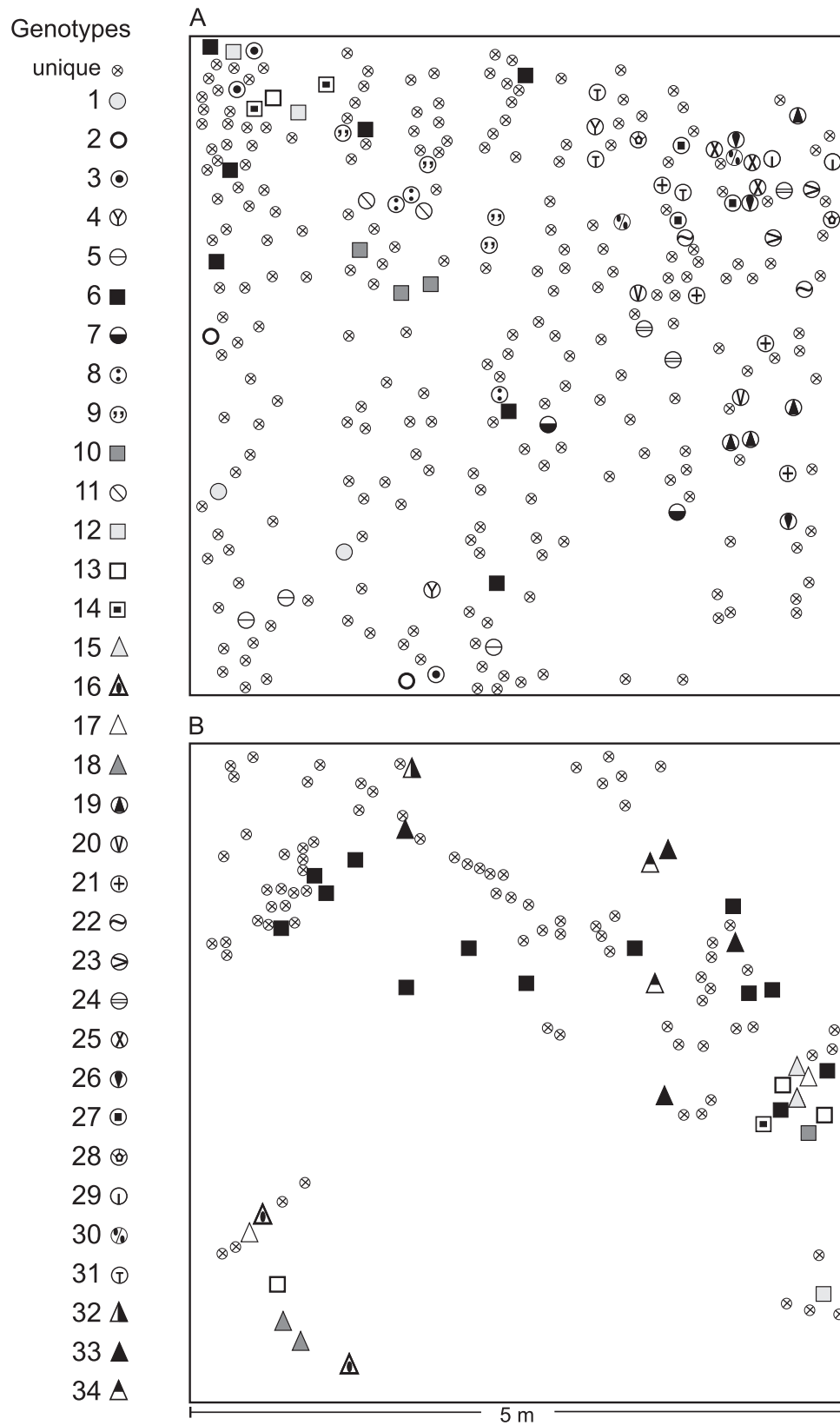


Fig. 2. Spatial distribution of genets and ramets within populations of *Filipendula ulmaria* at initial (A) and late (B) successional stage.

genetic differentiation between populations was significant ( $F_{ST} = 0.0078$ ,  $P < 0.05$ ; Table 3).

### 3.3. Pattern of spatial distribution of genets and ramets in populations

The spatial distribution of *F. ulmaria* genets in two populations was different, as it was demonstrated in maps (Fig. 2). The pattern of population B was close to clustered, and in population A to irregular with a tendency to ramet concentration. The range of variation in the ramet concentration of both same and different genotype was high. The comparison of distance between same genotype ramets demonstrated a high variation (Fig. 2). For example, they could be found within a distance of several centimetres (genets nos. 8, 9, 19, 27), within a 5–10 cm distance, or the distance between them could be up to 5 m (nos. 2, 3, 4, 5, 8, 26). Genets whose ramets were located in both populations, revealed the highest distance in the whole sample. In population B a tendency to the concentration of ramets was recorded both for genotypes present only in this population (nos. 15, 18) and present in the two populations. This was clearly demonstrated for one genet (no. 6) with the highest number of ramets (21), of which 13 were found in the population at the late stage, and 8 in the population at the initial stage of succession (Table 4).

Unique genotypes were dominant in both populations, and they formed large clusters. Many clusters were formed by ramets of various genotypes, including unique ones, which especially refer to population A (see the map Fig. 2). In both populations genets consisting of many ramets were rare, and they were most frequently formed by 2 ramets (in population A – 51%, and B – 60%, Table 2). Genotypes present in both populations were represented by 2 to 21 ramets when analysis was based on pooled data (A and B population), while within population they numbered 1 to 8 ramets per genotype in population A and 1 to 13 ramets in population B. Generally, ramets of different and unique genotypes formed a mosaic, and genets with packed ramets were rare, which was demonstrated in the maps (Fig. 2).

## 4. DISCUSSION

### 4.1. Relationship between genetic diversity, population age and ecological conditions

Studies on the mechanisms of genetic diversity of clonal species populations consider many features of long-lived plants which are decisive for different levels of genetic diversity, from  $D = 0.10$  to  $D = 0.90$  (Widen *et al.* 1994, Brzosko *et al.* 2002, Arens *et al.* 2005, Czarnecka and Ptaszyńska 2008). Our study demonstrated that populations of the same age (30 years) have the same level of diversity, although they developed under different stage of succession. Population B – at the late succession stage, has been developing along with transformation in vegetation cover and changing ecological conditions, from meadow to forest. Conditions for the growth of clonal species have been changing as shading increased and water level decreased during succession (Falińska 2003). After 25–30 years the population of *F. ulmaria* entered into a regression stage. At that time only 116 ramets grew on the plot, while 15 years before on the same 25 m<sup>2</sup> plot the number amounted to 838 (Falińska 1991). The decrease in abundance began when trees reached the age of 10 years. It was believed that in such circumstances only long-lived genets, adapted to modified conditions during succession from meadow to forest, would survive. Therefore, a high level of genetic diversity was not expected in populations which survived from the initial to the late stage of succession. This assumption was based on results of many publications which reported that when conditions become unfavourable for individual species, only a few genets have a chance to survive, and seedling recruitment is reduced by increased density, shading and competition (Slade and Hutchinsons 1987, Borkowska 2004). For example, it was reported that at the late succession stage, which was characterized with development of tree canopy and increase of shading, seedling emergence was limited and only 2–10% of ramets were fruiting in the population of *Maianthemum bifolium* (Honnay *et al.* 2006). Our study demonstrates that shading does not always result in such a high level of fruiting decrease at the late succession stage and that this effect is species-specific.

In the *F. ulmaria* population from the forest community, 45% of ramets were flowering and fruiting compared to 55% in the meadow population. Flowering ramets are located on the margin of tree biogroups which suggest that 'openwork' shading reduced the flowering considerably less than compact tree canopy in the forest. Also, the high proportion of unique genotypes (89%) in the forest population of *F. ulmaria*, indicates that the seedling recruitment process, despite the changing of abiotic and biotic factors during succession, can be described by the RSR pattern (Eriksson 1997).

#### 4.2. Factors determining the recruitment of seedlings during succession

Previous demographic studies of the population of *F. ulmaria* demonstrated that seedling recruitment was an irregular process when considering spatial dynamics of population-aspects, and that the changes in abundance sometimes fluctuated (Falińska 2003). Potentially, this may result from a permanent species replacement during succession, leading to the formation of gaps in vegetation cover and necromass on the abandoned meadow. Free spaces formed during the species replacement process, particularly dominants, are colonized by seedlings of *F. ulmaria* (Falińska 1991, 1996). Experimental studies by Borkowska (2004) carried out on the same area and involving the removal of plants and necrotic mass demonstrated that at the advanced succession stage micro-disturbances created an opportunity for seedling recruitment in clonal species populations. This hypothesis has also been confirmed in other studies (Stearns 1992, Watkinson and Powell 1993, Eriksson 1997). Grubb (1977) has defined gaps formed in such a process as 'regeneration niches', which not only ensure species abundance but also contribute to the genetic diversity of populations. This implies that large clusters of unique genotypes of *F. ulmaria* in meadow and forest populations (Table 2, Fig. 2) are formed as a result of the permanent colonization of gaps by seedlings in a long-term process of changes in the population and vegetation. Only one genotype replicated by 21 ramets was identified in the study. Potentially, it may have been a

fragment of one of the older genets which was present during the initial succession stage on the meadow. Four other genotypes shared by both populations were represented by a lower number of ramets (maximum 4 ramets per genotype), which does not rule out the possibility that they were also a remnant from the early stage of population development.

#### 4.3. Genetic diversity vs. prolonged clonal growth

The difference in the abundance of the *F. ulmaria* population at the initial and late succession stages was not fully reflected in different levels of genetic diversity. This study did not confirm the hypothesis on significant decrease in the level of genetic diversity along with the progress in succession. Results obtained from this study indicated that genetic diversity in population at the late succession stage resulted from a balance between sexual and asexual reproduction which, in the long-term dynamics of population, was maintained because of changed proportions between the number of progeny produced in both reproduction modes (Falińska 2003). A similar mechanism of genetic diversity is supported by the results obtained from the study on a meadow population of the same age, but formed in macroforbs (area of 0.6 ha), where succession was slower than in patches representing various succession stages in a mosaic plant cover on the area of 15 ha of abandoned meadows (Falińska 2003). The population in question was three times less abundant than the forest one. However, in both populations a high level of genetic diversity was estimated ( $D = 0.86$  for A and  $0.79$  for B population).

Former studies on the biology of *F. ulmaria* genets demonstrated that changes in some life history traits during their ageing could act as a mechanism that determine the genetic diversity of the population (Table 6). Although this species has a guerrilla growth strategy leading to the formation of a patched distribution pattern, its high level of genetic diversity was observed, as in other species (Ague *et al.* 2001, Wróblewska *et al.* 2003, Alberto *et al.* 2005). Different species can form patches which include one or several genets, as in populations of *Solidago*

Table 5. The relation between the age of genets and number of fragments after their disintegration and development of new genets (juveniles) within micro-gaps among the fragments. Data collected from marked genets during 20 years. Source: Falińska (1995), supplemented.

Genet	Age (years)	Area (m <sup>2</sup> )	Number of fragments	Number and men ramets/ fragments	New genets between fragments	
1	10	1.5	14	80	5.7±1.8	11
2	15	1.2	10	125	12.5±3.8	4
3	17	1.6	12	82	6.8±1.7	7
4	20	1.3	10	131	13.1±3.6	14
5	25	1.1	5	14	2.8±1.3	3

Table 6. Age-related variation in genet of *Filipendula ulmaria*. Data collected from marked genets during 20 years, the period 1980-2000. Source: Falińska (1995), supplemented.

Age class years	Number of genet (marked)	Percentage with senile parts	Participation of senile parts in genet (%)	Distance (cm) between ramet in genet	Disintegration of genet number
5-6	20	10	10.5± 1.6	4.0± 1.2	0
7-8	20	20	40.0± 2.7	8.3± 1.4	0
9-10	18	44	22.0±10.4	19.5± 3.1	5
11-12	16	75	41.3±29.8	20.8± 8.8	12
13-15	12	100	63.3± 6.4	35.8±28.8	10

*canadensis* (Hartnett and Bazzaz 1985), and *Maianthemum bifolium* (Arnes *et al.* 2005, Honney *et al.* 2006). It was demonstrated that a 400 m distance between populations or patches limits the gene flow, which explains the fact that in patches formed by the same species in forest communities the same genotypes are rarely (Arnes *et al.* 2005). The studied population of *F. ulmaria* were developed from initially abundant population across the entire meadow area (15 ha) immediately after the meadow was abandoned. Specimens of *F. ulmaria* grew in small clusters, which were distributed on the riverside or on the margin of the adjacent forest. A large patch of *F. ulmaria*, with almost regularly distributed ramets, was formed over 10–15 years after mowing ceased, and its disintegration began not earlier than after the tree biogroups occurred. After 30 years the forest community occupied 70% of the total area, and the remaining part was covered by patches formed by a few clonal species, also typical for meadow. Succession in mosaic vegetation cover was a non-uniform process. Therefore, the following issue may be considered: do the two populations, A and B, originate from one maternal population, which can be proven by shared genotypes, despite the 450 m distance between them and the mosaic of willow

brushwood and tree biogroups. The long-term survival of pioneering genets in populations was emphasized by Watkinson and Powell (1993).

A theory on the genetic variation of clonal plants has been documented in many studies (Eckert 1999). A review of 45 clonal species carried out in the past (Widen *et al.* 1994) demonstrated that among 21 species a single population was a monoclonal patch. Recently, after the introduction of DNA testing and improved methods for material sampling used in laboratory analysis, monoclonal plant populations of iterative growth are rarely found (Ague *et al.* 2000). A theory that prolonged clonal growth (Honnay and Bossuyt 2005) reduces sexual reproduction is under discussion, and scientists emphasize that the assessment of genetic diversity should be, to a greater extent, compared to the scale of the spatial and long-term dynamics of populations.

#### 4.4. Variation in life history traits throughout genet age as a mechanism of genetic diversity

Investigation of mechanisms that determine genetic diversity in populations of clonal species is mainly focused on the eval-

uation of ecological factors limiting seedling recruitment (Stearns 1992, Eriksson 1997, Fischer *et al.* 2004). It rarely considers changes in time of the biological features of genets. (Silvertown *et al.* 1997, Eckert 1999, 2002, Schmidt and Prati 2000). Such analysis is difficult, as genets/clones are long-lived and studies are, as a standard, carried out over several years, or if the research period is longer, the establishment of limits between genets is a considerable obstacle, as ramets multiply, partially die, and seedlings develop in the area occupied by genets simultaneously (Fig. 3). Over ten years a patch formed by a clonal species becomes a mosaic of several-year-old genets and unique, single-ramet ones, which has been demonstrated by a 15-year study of marked and mapped genets of *F. ulmaria* (Falińska 1995). The change in some traits, along with genet age, e.g. spreading and growth, vegetative multiplication, genet integration (Tables 5 and 6) and senescence that leads to disintegration, is reflected in seedling recruitment pattern RSR in populations of clonal species (Falińska 1995, 2003).

This correlation is illustrated by the biology of *Filipendula ulmaria* genets studied in the past (Falińska 1995). Genet development consists of four stages related to seedling recruitment. In the first period (1–5 years) the seedling develops into a rosette, and in the following seasons genets multiply the number of ramets from 25 to 60. They are featured by a monocentric structure and a round shape, and ramets are growing within a 2–3 cm distance form a compact cluster. In that period, seedlings (on average 2 to 6 m<sup>-2</sup>) are developing outside the area occupied by already established genets. In total, on a 25 m<sup>2</sup> plot about 56–77 seedlings are developed every season, of which 7–12% survive. Each season 5 to 11 new genets are formed. The second stage falls in the 6–7<sup>th</sup> year of development, when senescence of genets begins, which particularly refers to their central part and the peripheral multiplication of ramets. The largest genet, occupying an area of 1.5 m<sup>2</sup>, consisted of 122 ramets, and 10–20% of its surface was filled with necrotic mass, on which seedlings rarely developed. In that period 50% fewer seedlings occurred between genets than at the earlier stage (Fig. 3). A clustered pattern of the popu-

lation is transformed into a patched pattern, and the limits between young and old genets are invisible.

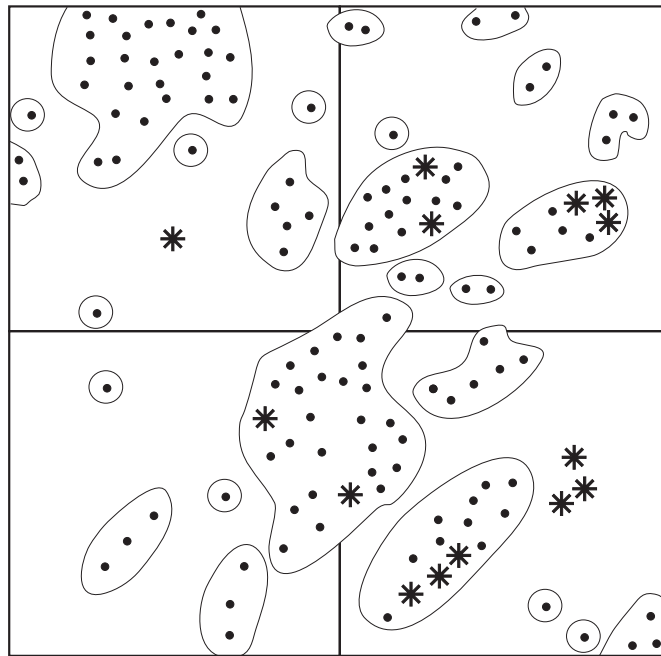
At the third (8–10 years) and fourth (10–15 years) stages ramets multiply and, simultaneously, 40–50% of the surface occupied by genets becomes covered by necrotic mass, which consequently leads to further disintegration of genets (Table 6). This is a crucial period for the domination of asexual over sexual reproduction. New genets are formed in spaces between fragments of genets and on their senile parts. This process is irregular and continues over the following years, to be reflected finally after 30 years in the domination of unique genets (Fig. 2). Over that period the curve representing the number of seedlings fluctuates, which correlates with the growth or senescence of genets, as well as with the increase or decrease in the number of ramets. This regularity has been emphasized in previous demographic studies on the populations of clonal species, carried out with a focus on the concept according to which population processes are observed on two levels: i.e. genets and ramets (Harper 1977, Harnett and Bazzaz 1985, Fair *et al.* 1999, Ague *et al.* 2001). Recently, many molecular methods have contributed to this concept, offering an opportunity to identify genets and their ramets (Falińska 2005, Arnaud-Haond *et al.* 2007). New opportunities are emerging for the assessment of demographic processes in populations of clonal species and for description of genetic diversity patterns, i.e. the distribution of genets and ramets in populations of plants with the ability to reproduce both asexually and sexually.

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after 10 year



after 20 year

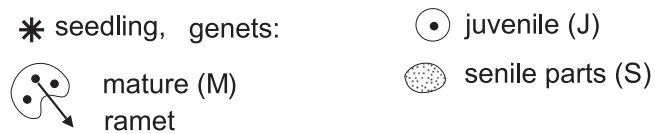
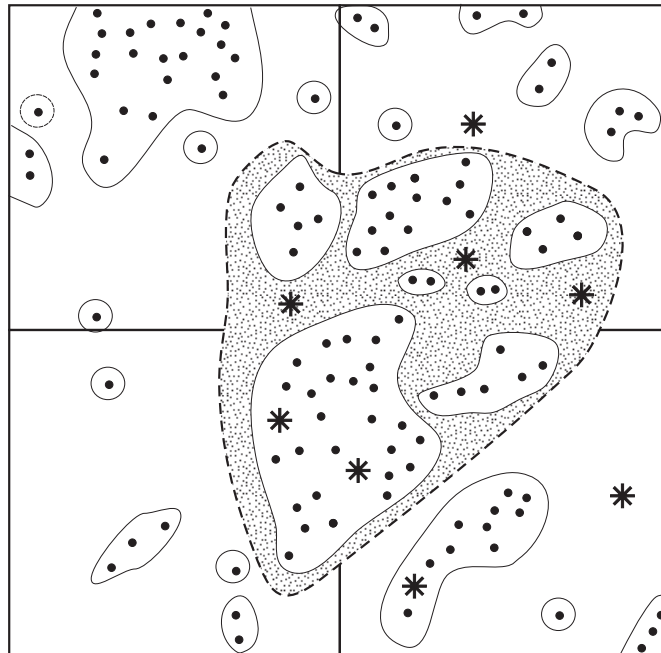


Fig. 3. Spatial distribution of seedlings and new genet (juvenile) within genets and micro-gaps among the fragments after genet disintegration and on their senile part in population *Filipendula ulmaria*. Data collected from plot  $2 \times 2$  m. Source: Falińska 1995, 2003.

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